For life science research only.
Not for use in diagnostic procedures.

Endoproteinase Lys-C Sequencing Grade

from *Lysobacter enzymogenes*

Version: 21
Content Version: June 2021

Lyophilized

Cat. No. 11 420 429 001	5 µg
Cat. No. 11 047 825 001	3 x 5 µg

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Endoproteinase Lys-C Sequencing Grade	Highly purified and specific protease. *A film of humidity occasionally present in the vials can be due to the strong hygroscopic nature of the lyophilizate. Stability and function of the enzyme are not influenced.*	11 420 429 001	1 vial, 5 µg
			11 047 825 001	3 vials, 5 µg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Endoproteinase Lys-C Sequencing Grade	Store at +2 to +8°C. ⚠ ***Store dry.***

1.3. Additional Equipment and Reagent required

For preparation of digestion buffer

See section, **Working Solution** *for additional information on preparing solutions.*

- Tris-HCl*
- EDTA

For solubilization of proteins

- Sodium dodecyl sulfate (SDS*)
- Urea
- Methylamine
- Guanidine hydrochloride
- Acetonitrile

1.4. Application

Use Endoproteinase Lys-C for the specific cleavage of proteins and peptides for:

- Protein structure
- Sequence analysis

The protease is suitable for digesting proteins in polyacrylamide gels.

2. How to Use this Product

2.1. Before you Begin

General Considerations

General handling recommendations

The content of one vial may be used for several simultaneous digests.
⚠ ***Take a new vial when repeating a digest in order to minimize the risk of contamination or autolysis.***

Activity determination

Activity determination of Endoproteinase Lys-C with Chromozym PL as substrate in the presence of stated concentrations of denaturing agents. Incubation of Endoproteinase Lys-C 200 µg/ml with denaturing agent for 6 hours at +25°C in 25 mM Tris-HCl buffer, pH 8.5, 1 mM EDTA.
ⓘ *Add 20 mM methylamine when applying urea.*

Denaturing agent	Concentration	Enzyme activity [%]
without addition (control)	–	100
SDS	0.001% (w/v) 0.01% (w/v) 0.1% (w/v)	113 136 109
Urea (+ methylamine)	0.1 M 0.5 M 1.0 M 4.0 M	122 106 90 86
Guanidine hydrochloride	0.1 M 0.5 M 1.0 M	60 27 12
Acetonitrile	1% (v/v) 5% (v/v) 10% (v/v)	122 157 161

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation/Composition	Storage and Stability	For use in...
Endoproteinase Lys-C Sequencing Grade	▪ Add 50 µl double-distilled water to the lyophilizate to a final concentration of 50 mM HEPES, pH 8.0, 10 mM EDTA, and 5 mg/ml raffinose. ⚠ ***To avoid autolysis, the incubation temperature must not exceed +37°C.***	Store 2 days at +2 to +8°C.	Digestion mixture
Digestion buffer	25 mM Tris-HCl* buffer, pH 8.5, 1 mM EDTA.	–	Dissolution of the proteins to be sequenced.

2.2. Protocols

Digestion of proteins in solution

See section, **Working Solution** *for information on preparing solutions.*

1. Dissolve the proteins to be sequenced in Digestion buffer.
   For proteins that are hard to solubilize, add urea, SDS, or guanidine hydrochloride to the Digestion buffer prior to solubilizing the protein. When applying urea, also add 20 mM methylamine.

2. Dilute protein solution with buffer, see section, **General Considerations** to achieve a suitable concentration of the denaturing agent in the digest.
   The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.

3. Choose an incubation time between 2 and 18 hours at +37°C, depending on the amount of enzyme.

Digestion of proteins in gels or on blotting membranes

See section, **Working Solution** *for information on preparing solutions.*

1. Dilute reconstituted protease solution with Digestion buffer to 1 to 5 µg Endoproteinase Lys-C in 100 µl.

2. Provide sufficient volume to the gel so that the gel is just covered or shrunken elements are reswollen.

3. Choose an incubation time between 2 and 18 hours at +37°C, depending on the amount of enzyme.

2.3. Parameters

Molecular Weight

33 kDa (reduced)
30 kDa (nonreduced)

Sequence

Sequence of Endoproteinase Lys-C

1		
GVSGSCNIDV	VCPEGNGHRD	VIRSVAAYSK
31		
QGTMWCTGSL	VNNSANDKKM	YFLTANHCGM
61		
TTAAIASSMV	VYWNYQNSTC	RAPGSSSSGA
91		
NGDGSLAQSQ	TGAVVRATNA	ASDFTLLELN
121		
TAANPAYNLF	WAGWDRRDQN	FAGATAIHHP
151		
NVAEKRISHS	TVATEISGYN	GATGTSHLHV
181		
FWQASGGVTE	PGSSGSPIYS	PEKRVLGQLH
211		
GGPSSCSATG	ADRSDYYGRV	FTSWTGGGTS
241		
ATRLSDWLDA	AGTGAQFIDG	LDSTGTPPV

3. Results

Verification of specificity and nonspecificity of Endoproteinase Lys-C

Endoproteinase Lys-C is a serine protease that specifically cleaves peptide bonds C-terminally at lysine in Tris-HCl buffer, pH 7.0 to 9.0. The specificity and nonspecificity of Endoproteinase Lys-C is verified using melittin as the substrate.

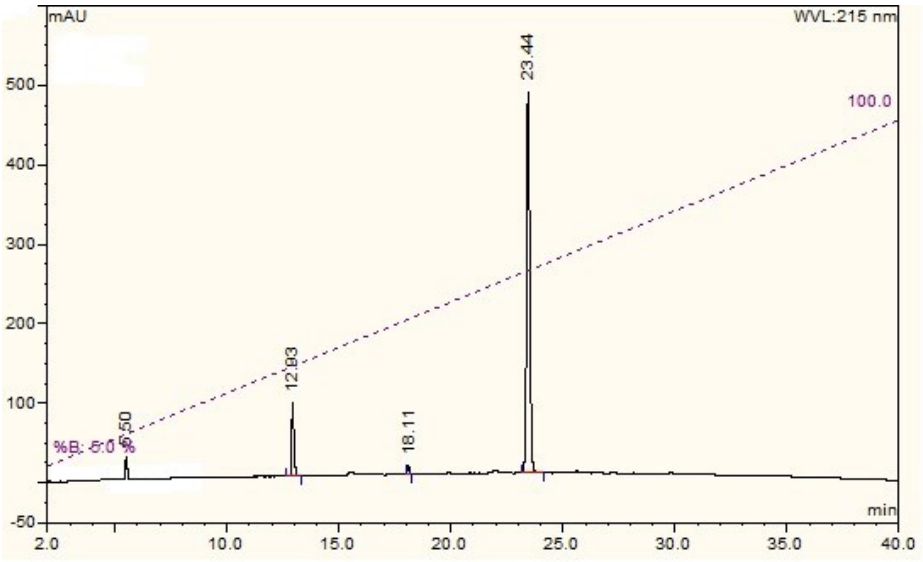

Fig 1: Specificity of Endoproteinase Lys-C in reversed phase HPLC. High concentrations of Endoproteinase Lys-C (1 part by weight enzyme with 10 parts by weight melittin) are incubated for 1 hour to detect the fragments of the specific digested substrate.

Digest	50 µg melittin in 100 µl 25 mM Tris-HCl, pH 8.5, 1 mM EDTA + 5 µg Endoproteinase Lys-C in 50 µl double-distilled water; 1 hour at +37°C; reversed phase HPLC Injection volume: 50 µl
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
Wavelength	215 nm
Fragments	5.50 min Arg(22) – Lys(23) and Arg(24) – Gln(26) 12.93 min Gly(1) – Lys(7) 23.44 min Val(8) – Lys(21)

3. Results

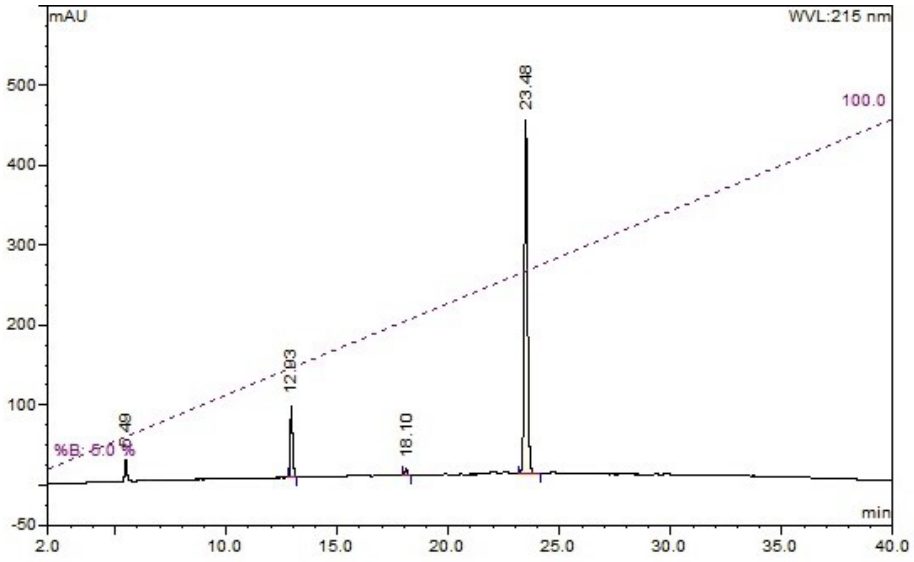

Fig 2: Nonspecificity of Endoproteinase Lys-C in reversed phase HPLC. High concentrations of Endoproteinase Lys-C (1 part by weight enzyme with 10 parts by weight melittin) are incubated for 18 hours to detect traces of impurities.

Digest	50 µg melittin in 100 µl 25 mM Tris-HCl, pH 8.5, 1 mM EDTA + 5 µg Endoproteinase Lys-C Sequencing Grade in 50 µl double-distilled water; 18 hours at +37°C; reversed phase HPLC Injection volume: 50 µl
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
Wavelength	215 nm
Fragments	5.50 min Arg(22) – Lys(23) and Arg(24) – Gln(26) 12.93 min Gly(1) – Lys(7) 23.44 min Val(8) – Lys(21)

4. Additional Information on this Product

4.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tris hydrochloride	500 g	10 812 846 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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